THE EFFECT OF pH ON THE MIDPOINT OXIDATION-REDUCTION POTENTIALS OF COMPONENTS ASSOCIATED WITH PLANT PHOTOSYSTEM II

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1. Introduction

Recent evidence suggests that the primary electron acceptor of plant Photosystem II is a specialized plastoquinone molecule and that the photoreduced acceptor is the unprotonated semiquinone anion [1–4]. Kinetic measurements indicate that the rate of proton uptake by chloroplasts is slower than the rate of oxidation by subsequent electron carriers of the photoreduced Photosystem II primary acceptor [5]. These kinetic results support the conclusion [1–4] that the primary acceptor functions between the oxidized form (A) and the unprotonated reduced form (A^-) rather than between the oxidized and the protonated reduced (AH) forms. As pointed out by Dutton and co-workers [6,7], the effective operating midpoint potential for such an A/A^-couple depends on the pK of the reduced form of the acceptor when oxidation–reduction titrations performed in equilibrium with hydrogen ion activity show a pH-dependent midpoint potential. These considerations apply to the primary acceptor of Photosystem II, which has been shown to have a midpoint oxidation-reduction potential that is pH dependent over the range from pH 6.0 to 8.6 [8,9]. It was therefore of interest to study the pH dependence of the midpoint potential of the Photosystem II acceptor over a wide range of pH so that the pK and effective operating potential could be estimated. Because the effect of pH on the midpoint potential of the Photosystem II acceptor could be studied most conveniently by monitoring the Photosystem II oxidation of cytochrome b_{559} at 77°K, the effect of pH on the oxidation–reduction properties of the cytochrome itself was also studied.

2. Methods

Signal: noise ratio considerations made it difficult to titrate directly the Photosystem II acceptor in untreated chloroplasts by following the *C*-550 absorbance change [9]. Estimation of the midpoint potential above pH 8.0 from titrations of the variable (light-induced) Photosystem II fluorescence at 77°K [10] was not possible because of quenching of the chloroplast fluorescence by the oxidation–reduction mediators in the alkaline pH region. Therefore, the oxidation state of the Photosystem II acceptor was monitored by measurement of the amount of cytochrome b_{559} photooxidized at 77°K [11] as a function of oxidation-reduction potential. Titrations of the variable fluorescence at 77°K at pH values below 8.0 gave midpoint potentials for the Photosystem II acceptor identical to those obtained by titrations of cytochrome b_{559} photooxidation at 77°K, in agreement with the results of Erixon and Butler [10] who showed that the Photosystem II photooxidation of cytochrome b_{559} at 77°K exhibits the same titration behavior as that of other indicators of the oxidation state of the Photosystem II primary acceptor.

The oxidation–reduction potential of samples to be assayed for cytochrome b_{559} photooxidation at 77°K was adjusted under anaerobic conditions with 0.02 M sodium dithionite or potassium ferricyanide, as described previously [12]. The sample was transferred by gas pressure to a low-temperature cuvette under an argon atmosphere and was frozen in liquid nitrogen. Cytochrome b_{559} photooxidation at 77°K was followed by measurement of the light-induced change in absorbance at 556 nm minus 540 nm with

dual wavelength spectrophotometer, as previously described [11].

Cytochrome b_{559} was titrated under aerobic conditions by measuring the cytochrome α-band spectrum of chloroplasts poised at defined potentials. The spectra were measured with an Aminco DW-2 spectrophotometer operated in the split-beam mode. To minimize contributions from cytochrome *f*, the oxidation state of cytochrome b_{559} was estimated from the absorbance difference at 561 nm minus 570 nm. The reference cuvette contained chloroplasts poised with potassium ferricyanide at $E_h > +500$ mV for the reductive titrations or chloroplasts poised with hydroquinone at $E_h < +300$ mV for the oxidative titrations.

3. Results

Fig.1 shows titrations of the low-temperature photooxidation of cytochrome b_{559} at two different pH values. The titrations are fully reversible, showing identical behavior in the oxidative and reductive directions. The curves drawn through the experimental points are theoretical one-electron curves with midpoints of −5 mV and −125 mV at pH 7.2 and pH 9.6, respectively. As the potential is lowered, the primary acceptor of Photosystem II becomes reduced prior to illumination. This prevents the photooxidation of *P*680, the reaction-center chlorophyll of Photosystem II [10,13] and the subsequent oxidation of cytochrome b_{559}, as indicated in the equations below.

$$(1)\ P680\cdot A \xrightarrow{h\nu} P680^{+}\cdot A^{-} \qquad P680^{+} + \text{cytochrome } b_{559} \rightarrow P680 + \text{cytochrome } b_{559}{}^{+}$$

$$\text{or } (2)\ P680\cdot A \xrightarrow{h\nu} \text{No reaction}$$

Fig.2 summarizes the results of midpoint potential determinations for the Photosystem II primary acceptor over the range from pH 6.5 to 9.6. It was not possible to maintain potentials stable enough to permit accurate titrations at pH values above 9.6. The line drawn through the experimental points is one for a component that takes up 1 H^+ per electron over the range from pH 6.5 to 8.9 and does not take up protons on reduction at pH values above 8.9 [14].

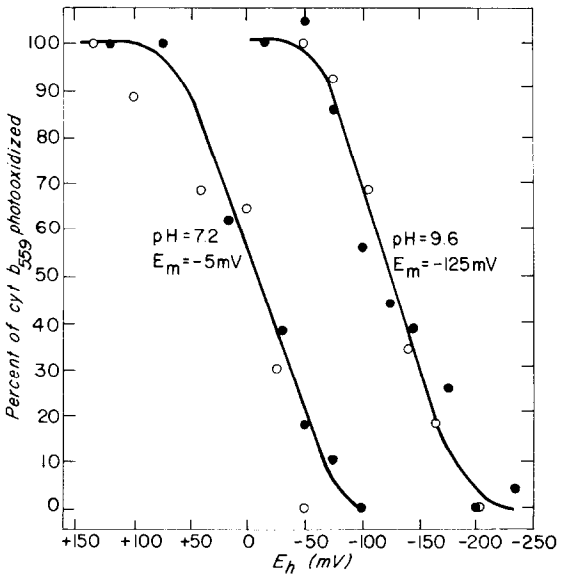

Fig.1 Effect of oxidation–reduction potential and pH on cytochrome b_{559} photooxidation at 77° K. The reaction mixture contained 20 mM NaCl, washed, broken chloroplasts at a chlorophyll concentration of 75 μM, and 100 mM potassium phosphate buffer (pH 7.2) or 100 mM glycine buffer (pH 9.6). The following oxidation–reduction mediators were added to facilitate equilibration between the platinum electrode and the bound chloroplast components: at pH 7.2, 1,2-naphthoquinone, phenazine methosulfate, phenazine ethosulfate, pyocyanine, 2-hydroxy-1,4-naphthoquinone, and anthraquinone-2-sulfonate at 5 μM. At pH 9.6, the mediator concentrations were increased to 7.5 μM and 7.5 μM 5-hydroxy-1,4-naphthoquinone, 7.5 μM duroquinone, and 2 μM indigotetrasulfonate were added. The open circles represent data from reductive titrations and the closed circles represent data from oxidative titrations.

The results shown in fig.2 indicate that the reduced form of the primary electron acceptor of Photosystem II has a p*K* of 8.9 ± 0.3. At pH values below the p*K* where the reduced primary acceptor is predominantly protonated, reduction of the unprotonated oxidized acceptor would result in the uptake of 1 H^+ per electron. At pH values above 8.9 where the reduced acceptor is predominantly unprotonated, reduction of the oxidized acceptor would not result in proton uptake. The pH-independent portion of the curve in fig.2 shows that the midpoint potential for the Photosystem II primary acceptor A/A^- couple is −130 mV (± 20 mV).

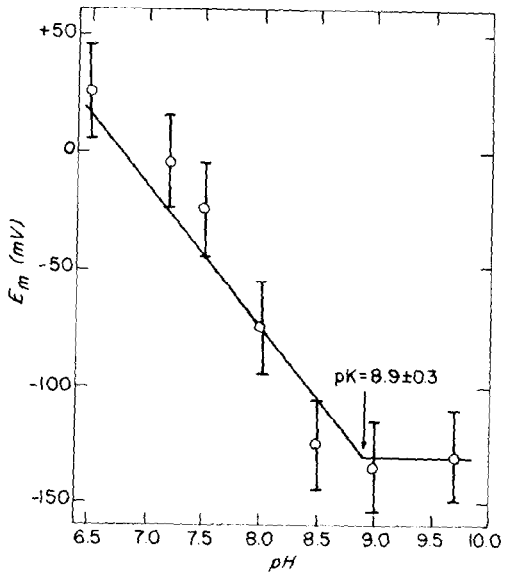

Fig.2. Effect of pH on the midpoint oxidation–reduction potential of the Photosystem II primary acceptor. Reaction conditions as in fig.1. Potassium phosphate buffer was used from pH 6.5 to pH 7.5, Tricine buffer, from pH 8.0 to pH 9.0, and glycine buffer, from pH 9.0 to pH 9.6. The mediator mixture described for the pH 7.2 experiment in fig.1 was used from pH 6.5 to pH 8.5, and the mediator mixture described for the pH 9.6 experiment was used for pH 9.0 to pH 9.6.

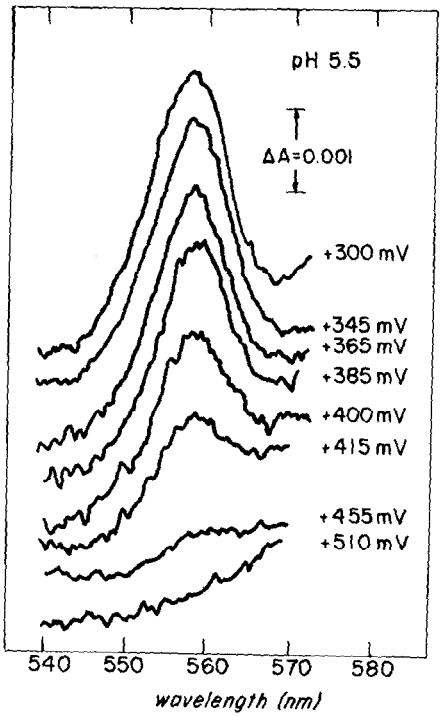

Fig.3. Oxidation–reduction titration of the high-potential chloroplast cytochromes. The reaction mixture contained 100 mM potassium acetate buffer (pH 5.5), 1 mM potassium ferricyanide, and washed, broken chloroplasts at a chlorophyll concentration of 50 μM. The potential of the reference cuvette was + 540 mV. Optical path-length, 1 cm. The potential of the reaction mixture, initially at + 540 mV, was lowered by the addition of small aliquots of 50 mM sodium ascorbate.

Because a reaction involving cytochrome b_{559} was the only method available for monitoring the effect of pH on the midpoint potential of the Photosystem II primary acceptor, it seemed important to investigate the effect of pH on the oxidation-reduction properties of the cytochrome itself. Fig.3 shows the experimental data from a typical reductive titration of cytochrome b_{559}. The data fit a one-electron titration curve with a midpoint potential of + 400 mV for cytochrome b_{559} at pH 5.5. Fig.4 summarizes the results of a series of oxidation-reduction titrations of cytochrome b_{559} over the range from pH 5.5 to 8.0. The experimental points represent an average of at least two oxidative and two reductive titrations at each pH. The data gave good fits for $n = 1$ titration curves at all pH values in this range. The titrations were completely reversible over this pH range, with midpoint potentials determined in the oxidative and reductive directions being equal within experimental uncertainty.

The midpoint potential of cytochrome b_{559} is + 375 mV (± 20 mV), independent of pH over the range from 6.0 to 8.0. These results are in good agreement with those of Bendall who obtained similar values between pH 6.5 and 7.5 in chloroplasts [15] and of Ikegami et al. [16] who found that the midpoint potential of the high-potential cytochrome b_{558} in *Euglena* fragments was pH-independent over the range from 6.0 to 9.0. At pH 5.5, the midpoint potential of cytochrome b_{559} was + 400 mV ± 5 mV. Identical results were obtained at pH 5.5 whether the buffer was potassium phosphate, potassium acetate, or sodium succinate. The amount of high-potential cytochrome b_{559} observed at pH 5.5 was identical to that observed at other pH values. No conversion of the

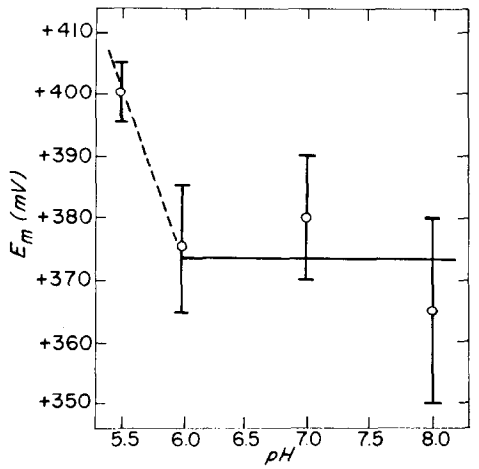

Fig.4. Effect of pH on the midpoint oxidation–reduction potential of cytochrome b_{559}. Potassium phosphate buffer was used from pH 5.5 to pH 7.5; potassium acetate and sodium succinate buffers were used at pH 5.5; and Tricine buffer was used at pH 8.0. For the reductive titrations, conditions were as described for fig.3. For the oxidative titrations, the initial potential was set with 500 μM hydroquinone and the potential was raised by the addition of small amounts of 200 mM potassium ferricyanide.

cytochrome to a lower potential at acid pH values of the type reported by Horton and Cramer [17] was observed.

These results suggest, as indicated by the lines drawn through the experimental points in fig.4, that cytochrome b_{559} may have a pK at pH 6.0 and take up 1 H^+ per electron at pH values below 6.0. (The dashed line in fig.4 represents the theoretical −60 mV per pH unit slope expected for such a result.) It was not possible to extend this curve by obtaining midpoint potentials at pH values below pH 5.5, probably because of the pronounced chloroplast coagulation that occurred in this pH region. Titrations at pH 5.0 showed n values that deviated substantially from one another and also exhibited considerable hysteresis, with midpoint values obtained in reductive titrations always approximately 50 mV more positive than those obtained in the oxidative titrations.

4. Discussion

The results reported above indicate that both cytochrome b_{559} and the primary electron acceptor of Photosystem II, two components involved in low-temperature Photosystem II reactions, have pH-dependent midpoint oxidation–reduction potentials. Of greatest significance is the finding that the reduced form of the Photosystem II primary acceptor has a pK of 8.9. Because the Photosystem II acceptor appears to function as an A/A^- couple rather than an A/AH couple over the time-scale of photosynthetic electron transfer reactions [1–5], a pK of 8.9 implies that the Photosystem II acceptor functions with an effective midpoint potential of −130 mV rather than the value near 0 mV determined at pH 7.0 (see above and [8,10]). A more electronegative midpoint potential for the Photosystem II acceptor would make oxidation of the photoreduced acceptor by the plastoquinone pool that functions as the secondary electron acceptor thermodynamically more favorable and would decrease the probability of a wasteful back-reaction with oxidized $P680$ of the type known to occur in Photosystem II at low temperatures [13,18,19].

An effective midpoint potential of −130 mV for the primary acceptor of Photosystem II might result in the release of sufficient energy on oxidation of the reduced acceptor by the plastoquinone pool to permit the coupled formation of ATP. This possibility should be considered in light of recent evidence for a site of ATP synthesis that involves Photosystem II and the plastoquinone pool [20,21]. Although the conclusion that cytochrome b_{559} has a pH-dependent midpoint potential must be considered to be somewhat tentative in the absence of data in the pH region below 5.5, the results reported above raise the possibility that the cytochrome may function as a proton carrier as well as an electron carrier. This may be of importance because the Photosystem II phosphorylation appears to require proton transport across the chloroplast membrane [22,23] and high-potential cytochrome b_{559} may play a role in electron transport near Photosystem II at physiological temperatures [24].

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